Citric Acid Cycle

Introduction and Features of the Krebs Cycle

The Krebs cycle, also called the citric acid cycle, also called the tricarboxylic acid **cycle** (TCA) is indeed a cycle. (We use these terms interchangeably; they are all common terms and all mean the same thing.) The Krebs cycle produces NADH and $FADH_2$. These compounds are then used by the electron transport chain to make ATP. As long as there is acetyl-CoA and oxygen, this cycle will continue. Although **no oxygen molecule** is required to further the cycle, the cycle would halt as NADH and $FADH_2$ accumulated. FAD and NAD are regenerated by the electron transport chain. NADH and $FADH_2$ are their counterparts made by the TCA cycle. The electron transport chain only works when there is oxygen.

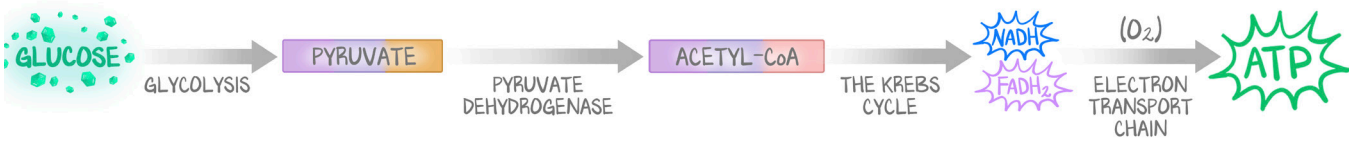

Figure 5.1: Glucose to ATP and All the Steps In Between
A global look at where we've come from and where we're headed.

The "purpose" of the TCA is to **burn acetyl-CoA to carbon dioxide**. This concept confuses people. We all spent hours memorizing every step back in college, and there's so much more to it than acetyl-CoA to CO_2. It has intermediate steps where products are neither created nor destroyed. Just like that game in the arcade, when you put the token in at the top, the wheel spins exactly 360°, ready for the next token, and your spent token lands at "CO_2."

The net equation is:

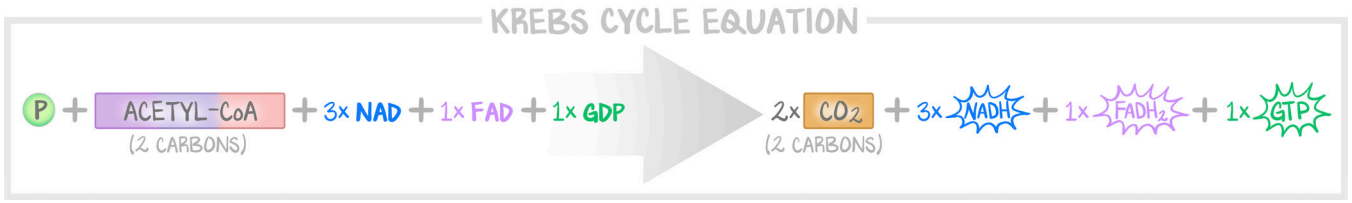

Figure 5.2: Krebs Cycle Equation
Basically, acetyl-CoA gets turned into CO_2... and some energy gets made. But it's that energy that is so crucial to cellular respiration and the generation of the much-needed ATP for cells to live.

Consider the profound nature of this statement. The cell respires—oxidative phosphorylation is called "cellular respiration." The whole point of the PDH, TCA, and ETC is to make high-energy intermediate compounds which will be turned into ATP. The way they are turned into ATP is by **using oxygen and making CO_2**. That is exactly what the body does at a macro level. The lungs breathe to remove CO_2 and to bring in oxygen. The heart and cardiovascular system are designed to bring oxygen to cells (with glucose) and pull away CO_2. Each cell gets energy by using oxygen and making CO_2, just as every human lives by using oxygen and making CO_2.

From a biochemical view, this is even more profound. None of the intermediate steps is present in this equation. Nothing is made. Nothing is consumed. As we will see in the nitty-gritty details to follow, and again later (in Metabolism #8: *Gluconeogenesis*) we are taught that we can *"exit this cycle to make glucose."*

Never can the citric acid cycle be exited. When acetyl-CoA goes in at the top, two CO_2 come out, and all the intermediate steps occur.

People say "enter" and "exit" because there are overlaps between the citric acid cycle and other metabolic pathways. Pyruvate can be used for gluconeogenesis through malate. Pyruvate can be used in fatty acid synthesis through citrate. While it's possible to see intermediaries of this cycle in **other pathways** (like gluconeogenesis), and often diagrams are made with an intersection of those pathways with the TCA, the TCA **never results in any product other than CO_2**. Acetyl-CoA can **never become glucose**. Acetyl-CoA can never become malate, oxaloacetate, citrate, or any other intermediary. Acetyl-CoA becomes CO_2. Along the way, energy gets picked up.

Generalities Before the Nitty-Gritty Details

The TCA is about formation of NADH and $FADH_2$ from NAD and FAD. The process by which NAD is turned into NADH or FAD is turned into $FADH_2$ is mediated by a **dehydrogenase**. Note that the terms "oxidation" and "reduction" have not been used. **Dehydrogenase** takes the NAD without an H, de-hydrogen-izes the thing in the cycle, and gives it to the NAD, making NADH. Every step that matters in this cycle is a dehydrogenase. The **name of the dehydrogenase** is the thing being dehydrogenated. That is, take the name of the substrate before the dehydrogenase step, add the word "dehydrogenase" to it, and that's the name of the enzyme. And everything is named appropriately…almost.

SUBSTRATE	ENZYME
Isocitrate	Isocitrate dehydrogenase
α-Ketoglutarate	α-Ketoglutarate dehydrogenase

SUBSTRATE	ENZYME
Succinate	Succinate dehydrogenase
Malate	Malate dehydrogenase

Table 5.1: Substrates and Their Enzymes
The only steps that matter are dehydrogenase steps. They are named a little funny.

But medicine can never be so simple, so there have to be exceptions. The only steps that matter are dehydrogenases. Dehydrogenases make NADH. **Except succinate**. Anything involving succinate works a little differently. First, **succinate dehydrogenase** is the only dehydrogenase that works with FAD to $FADH_2$. It is named correctly—succinate is the substrate and it dehydrogenases, but "it's a little different" because it makes $FADH_2$. Second, **succinyl-CoA synthetase** is named backwards. The step goes FROM succinyl-CoA to succinate, but is named as the succinate to succinyl-CoA. In this sense "it's a little different" because it's named backwards. Succinyl-CoA synthetase also is **substrate-level phosphorylation** making **GTP** for every turn of the cycle. Both of these enzymes involve succinate, and they happen to be next to each other in the cycle.

One acetyl-CoA enters, two CO_2 leave. They get spat out right away where CO_2 gets released. But the cycle still spins, and more energy is drawn from the cycle.

Specifics That Matter

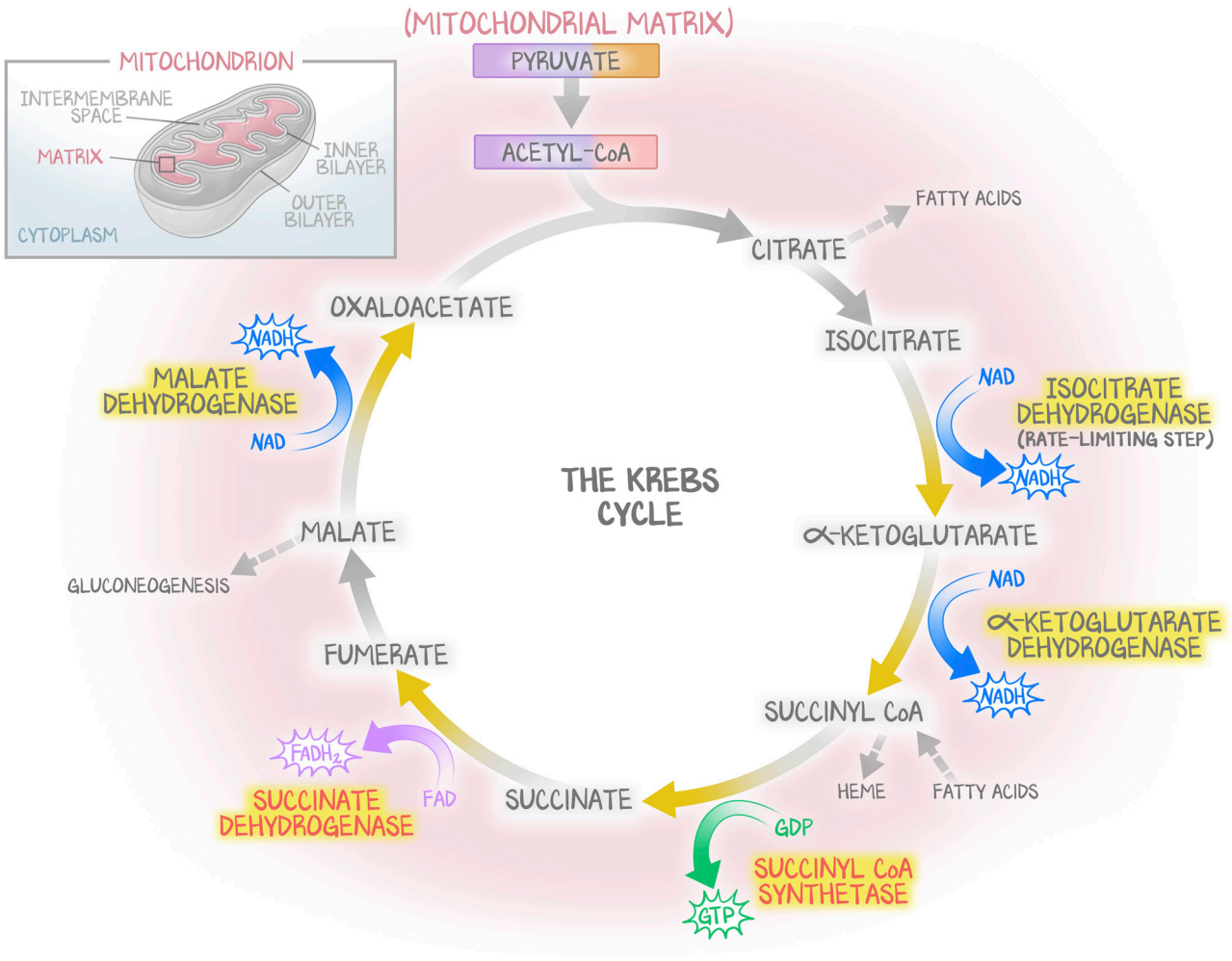

Figure 5.3: The Citric Acid Cycle
No need to memorize the entire citric acid cycle. The dehydrogenase steps are the energy-producing steps and the only ones that are medically important. The rate-limiting step is isocitrate dehydrogenase.

1. Oxaloacetate and acetyl-CoA combine to make citrate. Citrate becomes isocitrate.

2. Isocitrate turns into α-ketoglutarate. The thing that does that takes a hydrogen from isocitrate, and gives it to NAD. The enzyme that does this is **isocitrate dehydrogenase** (isocitrate, the thing giving up its H; and dehydrogenase, makes NADH from NAD). Isocitrate dehydrogenase is the **rate-limiting step** of the TCA and is the **target for substrate-level regulation**. Like pyruvate dehydrogenase, there is **no hormonal regulation** of the TCA. More on this later.

3. α-Ketoglutarate turns into succinyl-CoA. The thing that does this takes a hydrogen from α-ketoglutarate and gives it to NAD. The enzyme that does this is **α-ketoglutarate dehydrogenase** (α-ketoglutarate, the thing giving up its H; and dehydrogenase, makes NADH from NAD).

4. Succinyl-CoA becomes succinate [DING! DING! SUCCINATE!] through **succinyl-CoA synthetase**, and in doing so, makes **GTP**. We don't discriminate. GTP, ATP, all energy. So we're going to count this GTP as an ATP, when we keep track of "the count" at the end. This is special because it's a **substrate-level phosphorylation**. Special is a strong word, since we already had two of those at the end of glycolysis, but the boards like to use the word "substrate-level" to confuse you. This is the only other substrate-level phosphorylation discussed so far.

5. Succinate [DING! DING! SUCCINATE!] becomes fumarate. The thing that does this takes hydrogen from succinate and gives it to **FAD**. The enzyme that does this is **succinate dehydrogenase**. Succinate, the thing giving up hydrogen, and dehydrogenase (makes **$FADH_2$ from FAD**).

6. Fumarate goes to malate.

7. Malate turns into oxaloacetate (back at start!). The thing that does this takes a hydrogen from malate and gives it to NAD. The enzyme that does this is **malate dehydrogenase** (malate, the thing giving up its H; and dehydrogenase, makes NADH from NAD).

The Energy Count

For each acetyl-CoA the wheel turns once. Every turn gives us three NADHs, one GTP, and one $FADH_2$. Since every glucose molecule gives us two pyruvates, and every pyruvate gives us one acetyl-CoA, every glucose turns the cycle twice. The TCA cycle yields **6 NADH, 2 ATP** (GTP = ATP), and **2 $FADH_2$** per glucose.

The count was +2 ATP and +4 NADH. This brings the count to **+4 ATP, +10 NADH, and +2 $FADH_2$**.

Regulation of TCA

When acetyl-CoA enters the cycle, the wheel turns, and the cycle goes. There is **no hormonal influence**. The **rate-limiting step** is **isocitrate dehydrogenase**. It, like pyruvate dehydrogenase, requires thiamine, riboflavin, niacin, lipoic acid, and CoA to run.[1] Thiamine deficiency impairs the TCA. Most important is **substrate-level feedback**—just like pyruvate dehydrogenase. If **NADH accumulates**, the cycle will slow. Accumulation of NADH means that too much energy is being created, and the cycle should slow, OR it is a pathological state such as hypoxemia. Regardless, too much brake (NADH) and not enough gas (thiamine) are the major regulatory players of the TCA.

Entries and Exits and the TCA: Low-Yield Until You've Done All of Metabolism

Wait . . . we're saying this again? Yes. Because it is this important. In the Krebs cycle, the token goes in at acetyl-CoA. The cycle spins once, 360°, oxaloacetate to oxaloacetate. The cycle cannot be entered or exited. Substances are involved in more than one pathway. Parts of the TCA are the same as in other pathways. But just because substrates or reactions share the names of ones in the citric acid cycle does not mean the cycle can be entered at any point other than acetyl-CoA.

Glucose metabolism (glycolysis) makes pyruvate. Pyruvate can become acetyl-CoA. Pyruvate can be used for gluconeogenesis or fatty acid synthesis. Some amino acids break down to acetyl-CoA, others to oxaloacetate, some both. Fatty acid oxidation produces acetyl-CoA. Ketones produce acetyl-CoA.

Wherever acetyl-CoA is the final product, that product cannot become glucose. Acetyl-CoA is meant to generate NADH and $FADH_2$ for ATP (in the liver it can become ketones or fatty acids) through the TCA. Acetyl-CoA enters the cycle and the cycle goes one full turn.

If ever a breakdown results in any substrate of the TCA that is NOT acetyl-CoA, that substrate can be used for gluconeogenesis. For example, **odd-chain fatty acids** can be used for glucose generation; glucose generation intersects with the TCA as succinyl-CoA.

Succinyl-CoA is a high-energy intermediary that can be used for heme synthesis.

Malate can leave the mitochondria via the **malate shuttle**, a critical step in **gluconeogenesis** which can happen **only** in the **liver**.

Oxaloacetate leaves the cytoplasm as citrate for the citrate shuttle and lipid synthesis.

Citations

1. Source: Principles of Medical Biochemistry, 4th Ed, chapter 22 (https://www.clinicalkey.com/#!/content/book/3-s2.0-B9780323296168000220?scrollTo=%23hl0001267)